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Inhalation of swine-house dust increases the concentrations of interleukin-1 beta (IL-1β) and interleukin-1 receptor antagonist (IL-1ra) in peripheral blood

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Inhalation of dust in swine confinement buildings causes airway inflammation and systemic symptoms. The proinflammatory cytokines interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) increase in bronchoalveolar and nasal lavage fluid, and in serum. The aim of this investigation was to study changes in the IL-1 family of cytokines in peripheral blood in 36 healthy volunteers exposed to swine house dust for 3 h. Interleukin (IL-1β) was measured in platelet poor plasma and in a mononuclear cell fraction (PBMC) and interleukin-1 receptor antagonist (IL-1ra), IL-6 and TNF-α were measured in serum 4 and 7 h after the start of 3 h exposure. Lung function and a methacholine bronchial provocation test were performed before and 7 h after the start of exposure. The leukocyte count in whole blood and the mononuclear cell count in PBMC were examined before, and 4 and 7 h after the start of exposure. The concentration of airborne inhalable dust and endotoxin were measured using personal samples. The concentration of inhalable dust was 23 (20–30) mg m^{-3} (median 25th–75th percentile) endotoxin was 1·1 (0·8–1·4) μg m^{-3} and respirable dust (n=8) was 1·0 (0·7–1·2) mg m^{-3}. IL-1β increased from <0·125 to 0·9 (0·5–1·3) ng l^{-1} in plasma and from 1·6 to 2·7 (1·1–4·4) ng l^{-1} in PBMC (P<0·01). IL-1ra, IL-6 and TNF-α increased 2-, 12- and 2-fold in serum after exposure, respectively. Changes in IL-1ra correlated with changes in FEV_1, bronchial responsiveness, oral temperature (P<0·01) and blood white cell count (P<0·05). IL-1β correlated significantly with temperature (P<0·05). These results indicate that IL-1β and IL-1ra increase in peripheral blood following inhalation of swine house dust and may participate in and modulate the inflammatory response together with IL-6 and TNF-α.

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Introduction

Inhalation of large amounts of dust from a swine confinement building causes acute airways inflammation (1), increased bronchial responsiveness (2) and systemic reactions such as malaise, chills and fever (3). Cytokines like interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) increase in peripheral blood and in bronchoalveolar lavage fluid and may play a role in mediating local and systemic reactions. We have previously demonstrated increased levels of IL-1 (α and β) in bronchoalveolar and nasal lavage fluid (4), but we were not able to demonstrate increase in IL-1 in serum or plasma following exposure to swine dust. IL-1α and β are key cytokines in inflammatory reactions (5), and might be involved in the induction of systemic reactions together with IL-6 and TNFα. The mononuclear cell is one of the main producers of IL-1 in blood (6). We therefore wanted to study if airway exposure to toxic dust could lead to the induction of IL-1 production and release in peripheral blood by studying the freshly harvested mononuclear cell fraction from peripheral blood at different times after inhalation of swine dust. We also employed a slightly different method and a larger study group than previously in an effort to detect increase in IL-1β in platelet-poor blood plasma after exposure to swine dust.

The IL-1 receptor antagonist (IL-1ra) from the IL-1 family mainly functions as a downregulator of the inflammatory response (7). In this study we also measured changes in IL-1ra occurring in peripheral blood after dust exposure, and correlated changes in IL-1β or IL-1ra with changes in TNF-α, IL-6 and in peripheral blood leukocyte counts.

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Materials and Methods

SUBJECTS

The study involves 36 healthy volunteers (16 females), mean age 31 years (range 18–59 years). None had symptoms of

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allergy or asthma or had experienced respiratory infection within 2 weeks preceding the study. The mean FEV_1 was 99·2% (range 71–126) and vital capacity (VC) 97·3% (range 75–118) of predicted values. All subjects gave their informed consent and the local ethics committee approved the study.

STUDY DESIGN

Spirometry and measurement of bronchial responsiveness to methacholine were performed 3–14 days prior to exposure to swine dust. In the morning on the day of exposure, blood samples were drawn and oral temperature was measured. The subjects were then exposed for 3 h in a swine confinement building with approximately 700 swines weighing approximately 100 kg each. During this time a selection of swine was guided through a weighing box, a procedure that caused considerable aerosolization of settled dust. Each subject carried personal dust samplers throughout the whole exposure period.

Blood was drawn 3 h (n=8), 4 h (n=36), 7 h (n=36) and 24 h (n=8) after the start of exposure. Spirometry and a methacholine bronchial provocation test were performed 7 h after the start of exposure. The oral temperature was measured at 3, 5 and 7 h after the start of exposure and those who felt febrile or were uncertain about febrile symptoms continued to record their temperature at 9 and 11 h after the start of exposure.

LUNG FUNCTION AND BRONCHIAL RESPONSIVENESS

FEV_1 and VC were measured according to the ATS criteria (8) with a low-resistance rolling-seal spirometer (OHIO model 840, Airco, Madison, WI, U.S.A.). Local reference values were used (9,10). Bronchial responsiveness was measured with a methacholine provocation test. Inhalation of diluent was followed by doubling concentrations of methacholine starting at 0·5 mg ml^{-1} until FEV_1 had decreased 20% compared to the value obtained after inhalation of the diluent. The cumulative dose causing the 20% decrease in FEV_1 ($PD_{20}FEV_1$) was calculated. The method is standardized with regard to inhalation flow (0·4 l s^{-1}), inhalation volume (0·8 l), and number of breaths. The details of the procedure have been described elsewhere (11).

EXPOSURE MEASUREMENT

Inhalable dust was sampled at an air flow of 1·9–2·0 l min^{-1} with personal samplers using 25 mm IOM head open-phase filter cassettes and IOM air suction pumps (SKC Ltd, Dorset, U.K.). The cassettes were carried in the breathing zone and were equipped with 0·4 mm polycarbonate filters (Nuclepore®, Costar Corp Headquarters, Cambridge, MA, U.S.A.). Eight subjects carried Cyclone® samplers (25 mm 8 mm Millipore SCWP) for measurement of the respirable dust fraction. Inhalable dust and respirable dust were measured by weighing after 24 h of conditioning, using a Mettler® ME22 balance (Mettler, Greisensee, Switzerland) and reference filters. Endotoxin in the inhalable dust was measured after extraction in suitable dilution with a chromogen version of the limulus amebocyte lysate assay (CoA endotoxin test, Kabi Vitrum Diagnostic, Stockholm, Sweden, with *Escherichia coli 0111* B4 as standard). The method has been described in detail elsewhere (12).

CYTOKINE ASSAYS IN SERUM

IL-1ra, IL-6 and TNF-α were analysed using commercial kits (Quantikine® R&D systems, Europe Ltd, U.K.). IL-1ra and IL-6 were determined using a sandwich enzyme immunoassay technique with a monoclonal antibody. The lowest standard for IL-1ra and IL-6 were 46·9 and 3·9 ng l^{-1}, respectively. TNF-α was measured with the high sensitivity sandwich enzyme immunoassay method from the same source. The lowest standard was 0·5 ng l^{-1}. All assays were done in duplicate. Samples with a coefficient of variation (CV) <10% were not accepted.

ISOLATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS

Peripheral blood mononuclear cells (PBMC) were separated from sterile EDTA blood (4·5 ml, Becton Dickinson, San Jose, CA, U.S.A.) by centrifugation through Histopaque-1077 (Sigma Diagnostics, St. Louis, MO, U.S.A.) (n=8) or Ficoll–Paque (Pharmacia, Uppsala, Sweden) (n=8). In the histopaque method 3 ml blood was applied on 3 ml histopaque in a polypropylene sterile conical centrifuge tube. In the Ficoll–Paque method 2 ml blood was mixed with 2 ml of balanced salt solution and applied on 3 ml of Ficoll–Paque in a sterile glass tube. Subsequent steps were identical in both methods. The samples were centrifuged at 400 g for 40 min at room temperature. The interface cells were then harvested, mixed by gentle aspiration, washed twice with Hank's balanced salt solution (Biochrom KG, Berlin) and centrifuged at 250 g for 10 min. Two portions of each sample were prepared.

One portion consisted of the washed cell pellet that was resuspended in 0·5 ml Hank's solution (only histopaque separated blood, n=8). A sample was spread on a film dried and stained with May–Grünwald–Giemsa stain. Five hundred to 700 cells were counted using light microscopy. The purity of the sample was checked and the total PBMC cell count and the proportion of lymphocytes and monocytes were counted. Viability of PBMC was determined with the trypan blue exclusion test and was more than 95%.

The second sample consisted of the washed cell pellet resuspended in 3 ml Hank's solution (Histo-Paque sample), or 2 ml Hank's solution (Ficoll–Paque) and was stored in −70°C. This sample was freeze–thawed two cycles to release intracellular IL-1β before analysis.

PREPARATION OF PLATELET-POOR PLASMA

Blood samples were collected in sterile vacuum blood collection tubes containing EDTA. The tubes were centrifuged

immediately after venipuncture (400–*g* for 10 min). Plasma was removed without disturbing the buffy coat, aliquoted in 1·5 ml microfuge tubes and was spun at 1600 *g* at 3000 rpm for 10 min to pellet the platelets. The platelet free plasma was transferred to new microfuge tubes and frozen until assay.

IL-1β ASSAY IN PBMC AND PLASMA

The concentration of IL-1β in the freeze–thawed PBMC sample was measured in 16 subjects. In eight of these subjects extra blood samples were taken at 3 h and 24 h, in order to study the time course of IL-1β release. In the other eight subjects both the IL-1β in PBMC was measured and the proportion of monocytes in the PBMC fraction.

IL-1β was measured in platelet poor plasma (n=8) using the high sensitivity Quantikine® commercial ELISA kit (Europe Ltd, U.K.). The lowest standard was 0·125 ng l^{-1}.

PERIPHERAL BLOOD CELL COUNTS

Peripheral blood was collected using EDTA tubes and was rolled for 30 min. The sample was prepared using the COULTER Q-PREP® to obtain leukocytes with the cell surface markers CD45/CD14 and was analysed using flow cytometry (EPICS® PROFILE II, Coulter Electronic Ltd, Northwell Drive, Luton, Bedfordshire, U.K.).

STATISTICAL ANALYSIS

The results were analysed using Stat-View, 4.0 (Abacus concepts Inc). Wilcoxon's signed rank test, two-tailed was used for comparing paired observations. Spearman ρ (rho) were used to estimate the correlation of IL-1β and IL-1ra with exposure and health effects. Correlation for multiple comparisons was made with the Bonferroni method. Data were expressed as median (25th–75th percentile) or mean ± SEM unless otherwise stated.

Results

The concentration of inhalable dust in the swine house during exposure was 23 (20–30) mg m^{-3}. The concentration of respirable dust (n=8) was 1·0 (0·7–1·2) mg m^{-3}.

TABLE 1. Lung function and oral temperature before and 7 h after the start of exposure to swine dust in 36 healthy subjects (median, 25th–75th percentiles)

	Before exposure	After exposure
FEV_1 (l)	3·8 (3·5–4·9)	3·6 (3·3–4·6)*
VC (l)	4·8 (4·3–6·0)	4·6 (4·1–5·8)*
$PD_{20}FEV_1$ (mg)	2·7 (1·1–11·2)	0·3 (0·1–0·7)*
Temperature (°C)	36·2 (36–36·5)	36·7 (36·5–37·1)*

*P<0·01.

The concentration of endotoxin (inhalable dust fraction) was 1·1 (0·8–1·4) $\mu g\ m^{-3}$.

The oral temperature increased 0·5 (0·5–0·6) °C at 7 h after the start of exposure. Five subjects had an oral temperature above 38°C, at 9 h or later after the start of exposure.

FEV_1 fell by 6% and VC by 3% compared to the pre-exposure value (P<0·01). $PD_{20}FEV_1$ for methacholine fell from 2·7 (1·1–11·2) to 0·3 (0·1–0·7) mg (P<0·001). The lung function, challenge test and temperature are shown in Table 1.

LEUKOCYTES IN BLOOD

The concentration of granulocytes in peripheral blood increased in all subjects after exposure and reached maximum levels at 7 h after start of exposure. Peripheral blood monocytes increased, but lymphocytes decreased (Table 2).

In the mononuclear cell fraction (n=8), the number of lymphocytes decreased from 2·3 ± 0·2 to 1·6 ± 0·2 × 10^9 cells l^{-1} and the monocytes increased from 0·5 ± 0·1 to 0·7 ± 0·1 × 10^9 cells l^{-1} 7 h after the start of exposure (Fig. 1).

IL-1ra, IL-6 AND TNF-α IN SERUM

IL-1ra increased significantly from 222 (123–297) ng l^{-1} to 316 (196–446) ng l^{-1}, and 547 (398–657) ng l^{-1} at 4 and 7 h after the start of exposure, respectively (Fig. 2).

The IL-6 concentration increased from less than 1·5 ng l^{-1} to 14·8 (7·2–25·5), and 18·9 (7·9–34) ng l^{-1}

TABLE 2. Leukocyte concentrations in peripheral blood measured with flow cytometry before and after exposure to swine dust. Mean ± SEM

	Before exposure	After exposure	
		7 h	24 h
Granulocytes × 10^9 cells l^{-1}	2·3 ± 0·1	6·6 ± 0·3*	3·9 ± 0·2*
Lymphocytes × 10^9 cells l^{-1}	1·7 ± 0·1	1·4 ± 0·1*	1·5 ± 0·1*
Monocytes × 10^9 cells l^{-1}	0·35 ± 0·02	0·51 ± 0·03*	0·39 ± 0·02

*P<0·001 compared with pre-exposure values.

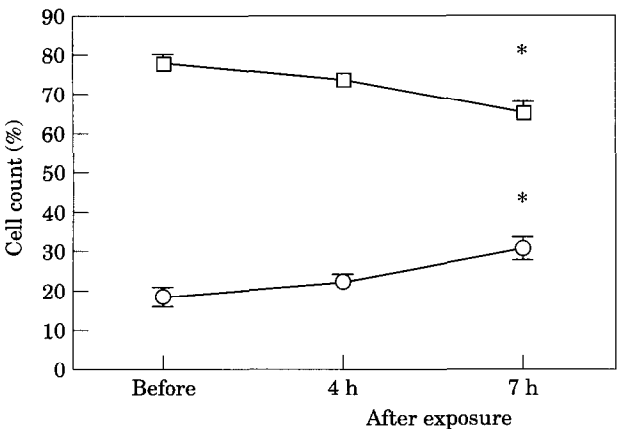

FIG. 1. Percent lymphocytes (□) and monocytes (○) in the PBMC fraction before, and 4 and 7 h after, the start of 3 h exposure to swine dust. Mean ± SEM (n=8). *P<0·02 compared with pre-exposure values.

at 4 and 7 h after the start of exposure, respectively (Fig. 2).

The TNF-α concentration increased from 1·8 (1·4–2·7) ng l^{-1} to 3·4 (2·1–5·2), and 2·5 (1·6–3·3) ng l^{-1} at 4 and 7 h after the start of exposure, respectively (Fig. 2). All changes were statistically significant at P<0·01.

IL-1β in PBMC AND PLASMA

The concentration of IL-1β in PBMC was significantly higher at 3, 4 and 7 h but not at 24 h after the start of exposure compared to pre-exposure values (Fig. 3). Detectable levels of IL-1β were found in platelet-poor plasma at 4 and 7 h after the start of exposure, but not prior to exposure (Table 3).

CORRELATIONS

Endotoxin in inhaled dust correlated with increase in IL-1β in the PBMC fraction (Spearman ρ 0·64, P<0·05). The concentration of respirable dust correlated with increase in IL-1β in plasma (Spearman ρ 0·96, P<0·008, n=8).

IL-1ra in serum correlated negatively with $PD_{20}FEV_1$, and positive with temperature changes, leukocyte count (Table 4, and TNF-α (Spearman ρ 0·74, P<0·001) and IL-6 (Spearman ρ 0·62, P<0·005) in peripheral blood.

Discussion

Inhalation of swine confinement building dust induces not only a local inflammation in the airways, but also systemic symptoms and signs including fever and malaise, rise in acute phase proteins and peripheral blood granulocytes (1,4). These findings suggest increased blood concentrations of proinflammatory cytokines, like IL-1, IL-6 and TNF-α. In previous communications we have reported an early appearance of TNF-α in peripheral blood, and an IL-6 response with a slower and more protracted time course.

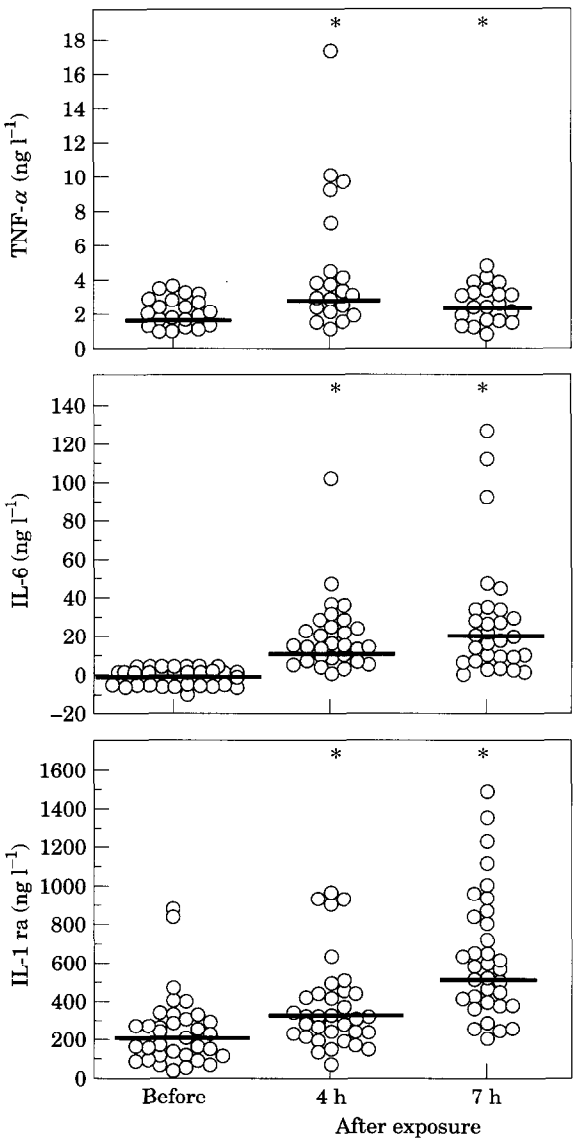

FIG. 2. TNF-α (n=21), IL-6 (n=32), and IL-1ra (n=36) in serum before and after exposure to swine dust. *P<0·001 compared with pre-exposure values. Horizontal bars denote median value.

We did not mention IL-1, since we were unable to demonstrate it in peripheral blood. However, due to methodological improvements we can now report increased, but still rather low levels of IL-1β, in peripheral blood of volunteers exposed to swine dust. We also report an increase in IL-1ra in peripheral blood.

We tested three methods for the analysis of IL-1β in blood: the chloroform extraction method, use of fresh platelet-poor plasma (13), and separation of peripheral blood mononuclear cells (14). IL-1β was measured with an amplified ELISA method. The chloroform method gave the same results (not reported) as the platelet-poor plasma method.

A delay in the separation of plasma from blood or platelets from plasma may interfere with the analysis of

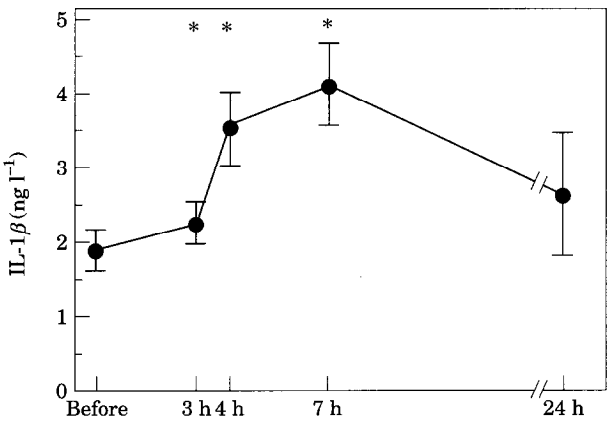

FIG. 3. IL-1β concentration in the PBMC fraction at different times after exposure to swine dust (Ficoll–Paque method). *P<0·01 compared with pre-exposure values. Mean ± SEM.

TABLE 3. IL-1β in PBMC (n=16) and in platelet-poor plasma (n=8), median (25th–75th percentiles)

	Before exposure	After exposure	
		4 h	7 h
PBMC (ng l^{-1})	1·6 (1·2–1·8)	2·5 (1·8–3·2)*	2·7 (1·1–4·4)*
Plasma (ng l^{-1})	<0·125	0·7 (0·4–0·9)*	0·9 (0·5–1·3)*

*P<0·01 compared with pre-exposure values.

TABLE 4. Correlations between changes in IL-1ra (n=36) in serum or IL-1β (n=16) in PBMC 7 h after start of exposure and change in lung function, oral temperature and leukocytes in peripheral blood

	IL-1ra		IL-1β	
	ρ	P†	ρ	P
FEV_1	−0·55	0·002**	−0·61	0·042
$PD_{20}FEV_1$	−0·56	0·001**	−0·54	0·047
Temperature‡	0·71	<0·001**	0·77	0·011*
Granulocytes	0·52	0·012*	0·63	0·036

ρ, Spearman rho.
†According to the Bonferroni correction, P<0·0125 is required for significance at the 5% level and P<0·0025 at the 1% levels.
‡n=32.
*P<0·05, **P<0·01.

IL-1, due to clotting factors and proteolytic enzymes (15,16). We therefore used freshly prepared platelet-poor plasma. The level of IL-1β in plasma was below the detection limit of the amplified assay in samples collected before exposure. After exposure however, we observed a small increase of IL-1β in plasma into the range where it could be measured. These levels are compatible with results reported by other authors using the same assay in studies on patients with silicon implants or with ovarian stimulation syndrome (17,18).

The PBMC fraction, that was freeze–thawed twice in order to release intracellular IL-1β, and had about four times higher concentration of IL-1β compared with plasma. The plasma and PBMC fraction values are, therefore, difficult to compare. The contribution of intracellular IL-1β may have increased the concentration of IL-1β in the PBMC fraction (19). The presence of soluble IL-1 receptor in plasma may have resulted in lower availability of immunoreactive IL-1β in the plasma fraction (20).

The increase in IL-1β in the PBMC fraction was modest (about 70%). Since the number of monocytes in peripheral blood also increased about 50%, part of the change in IL-1β in the PBMC fraction might be accounted for by the presence of more monocytes containing IL-1β, rather than by increased IL-1 synthesis. On the other hand, the increase in IL-1β in platelet-poor plasma suggests a true increase in secretion of IL-1β. This is also anticipated since TNF-α induces IL-1β production in monocytes and there was a clear increase in serum TNF-α that clearly preceded maximum values for IL-1β (21). The correlation between increased endotoxin concentration in respirable dust and IL-1β in plasma suggest that the changes were induced by some agent(s) in the swine house air.

We noted an almost 200-fold higher concentration of IL-1ra in serum compared to IL-1β, which should be sufficient to block many of the effects of IL-1β. Intravenous infusion of endotoxin or IL-1 induces a 10- to 40-fold excess of IL-1ra over IL-1β (22). Thus it is possible that other agents cause part of the IL-1ra excess, such as IL-6 or GM–CSF (granulocyte macrophage–colony stimulate factor) (not measured), which are known to release IL-1ra, but not IL-1β (23).

The data gives little support to the concept that inhaled endotoxin may reach the peripheral circulation, since this should have induced a more intense activation of blood monocytes, and caused a clear increase in IL-1β (24). TNF-α and IL-6 are probably important mediators of the peripheral effects observed after inhalation of swine dust (25,26). The role of IL-1β in peripheral blood is uncertain. Thus the concentration of IL-1β was low, and the concentration of IL-1ra was high, but perhaps even very low concentrations of IL-1β may induce significant effects in co-operation with other cytokines.

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